AN ELECTROPHORETIC STUDY ON STRUCTURAL COMPONENTS OF *MICROCOCCUS LYSODEIKTICUS*

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SUMMARY

Suspensions of intact bacteria, protoplasts, protoplast membranes and the derived membrane lipid and defatted membrane fractions of *Micrococcus lysodeikticus*, as well as cardiolipin, have been studied by micro-electrophoresis. The pH–mobility relationships and the influence on surface charge density of thorium and uranyl ions were used to characterize the ionogenic groups present on the surfaces. For this purpose the concept of isosteric ion adsorption concentrations is preferred in these systems to the use of ionic reversal of charge concentrations. For bacteria, protoplasts, protoplast membranes and defatted protoplast membranes, the electrophoretic behaviour is controlled by the ionization of carboxyl and amine groups in the surface, whilst the membrane lipid and cardiolipin are phosphate type colloids. The results provide experimental evidence to support the view that in the structure of the protoplast membrane, the lipid occupies an internal position sheathed by protein and possibly carbohydrate.

INTRODUCTION

Studies[1] on model systems consisting of known natural colloids have shown that each type of ionogenic group has a characteristic electrophoretic behaviour pattern and a characteristic sequence has been found for the concentrations of various ions required to reverse its charge, which is termed an "ion spectrum". In particular, thorium ions have been found to be more effective than uranyl ions in reversing the charge of carboxyl type colloids, but not for some phosphate types.

In recent years, micro-electrophoretic methods[3] have been used to investigate the electrokinetically active surface layers of several bacteria. In the present paper, the effects of hydrogen, uranyl and thorium ions on the electrophoretic properties of a number of components from *M. lysodeikticus* and its protoplasts are presented in order to characterize the types of ionogenic groups present in their surfaces and to provide information on protoplast membrane structure.

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METHODS

Materials

The organism used was *Micrococcus lysodeikticus* (N.C.T.C. 2665) grown on beef tryptic digest medium as in earlier work[3]. Electrophoretic measurements were made on suspensions of intact bacteria, protoplasts, protoplast membranes and the lipid and defatted membrane fractions derived from the protoplast membranes. The preparation of each of these has been described previously[3]. In addition, a sample of cardiolipin was kindly supplied by Dr. M. PANGBORN. Reagents were of A.R. quality.

Micro-electrophoresis

All measurements were made at 25° in a horizontal cylindrical glass micro-electrophoresis cell using methods previously described[4]. Most observations of particles were made under dark ground illumination. For any reading, ten observations were made, five in each direction, so as to minimise the effect of small random drifts.

Suspensions were prepared by shaking in 0.05 *M* NaCl. No buffer solutions were used because of possible specific ion interactions. To obtain pH-mobility relationships, the pH of each suspension was adjusted over the range 1 to 11 with small quantities of 0.05 *M* HCl and 0.05 *M* NaOH. Other measurements were made on suspensions in the presence of uranyl nitrate and thorium nitrate at a number of concentrations. Where necessary, the concentration of NaCl was reduced to maintain constant ionic strength. With the more concentrated uranyl nitrate solutions, the NaCl was replaced with NaOAc to reduce the lowering of pH produced by hydrolysis. Protoplasts had to be examined in the presence of isotonic sucrose (1 *M*) and, for comparison, some measurements on protoplast membranes were made in the same medium.

RESULTS

pH-mobility curves

The measured mobilities were converted to zeta potentials by means of the HELMHOLTZ-SMOLUCHOWSKI equation for large particles

$$\zeta = \frac{4\pi}{D}\eta \cdot v \qquad (1)$$

where η and D are the viscosity and the dielectric constant, within the electric double layer, assumed to be those for water, and v is the electrophoretic mobility. The pH of the surface at the plane of shear was then calculated from the equation of HARTLEY and ROE

$$pH_s = pH_{Bulk} + \frac{e\zeta}{2.303kT} \qquad (2)$$

where e is the electronic charge, k is the Boltzmann constant and T the absolute temperature.

In Fig. 1, the electrophoretic mobility has been plotted against pH_s for each system. The protoplasts and protoplast membranes showed identical behaviour in 1 *M* sucrose. Chiefly due to the effects of viscosity, the mobility values of membranes in 1 *M* sucrose, 0.05 *M* NaCl, were about one fifth of the corresponding mobilities in 0.05 *M* NaCl. It was concluded that protoplasts and the membranes derived from them possess similar electro-kinetic properties. The experimental difficulties arising

from the lysis of protoplasts made it preferable to study protoplast membranes instead of intact protoplasts.

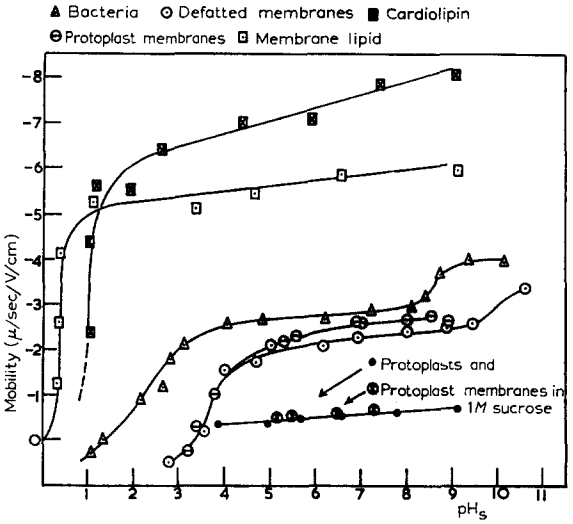

Fig. 1. pH_s-mobility curves of suspensions in 0.05 *M* NaCl of cardiolipin and bacterial derivatives.

p*K* values for the surface ionogenic groups have been derived from the curves in Fig. 1. The surface charge densities corresponding to the ionized and the un-ionized form of each group, revealed by the plateaux in the mobility–pH_s curves, were calculated using the GOUY-CHAPMAN equation

$$\sigma = 3.53 \cdot 10^4 \, c^{\frac{1}{2}} \sin h \frac{e\zeta}{2kT} \qquad (3)$$

where c is the concentration of uni-univalent electrolyte. Each mobility corresponding to the mean of the ionized and unionized charge densities was then estimated and the corresponding value of pH_s obtained. The values of p*K* so derived are approximate because the surface ionogenic groups may not be precisely in the plane of shear. Further, where more than one type of charge group is present, the charge densities considered should strictly relate to conditions under which charge groups not under consideration are unionized.

Results are given in Table I. With bacteria and defatted protoplast membranes there appear to be both acidic and basic ionogenic groups in the surface. Protoplast

TABLE I

p*K* VALUES FROM ELECTROPHORETIC DATA ON CELL FRACTIONS

System	pK_A	pK_B
Bacteria	2.1	8.6
Protoplast membranes	3.6	—
Defatted membranes	3.6	9.95
Membrane lipid	0.4	—
Cardiolipin	1.05	—

membranes probably possess a basic group similar to the defatted membranes although, due to disruption of the membranes in alkaline media, it was not possible to demonstrate this experimentally. The membrane lipid is simpler in that it exhibits only one very acidic type of ionogenic group. For comparison, cardiolipin has been used as a purely phosphate type of colloid. The two lipids show a similar type of behaviour, although the membrane lipid has a significantly lower pK.

Charge groups and ion adsorption

The effects of the adsorption of uranyl ions and thorium ions on the colloid surfaces have been compared using surface charge densities calculated from mobility measurements by means of eqns. (1) and (3). The reduction in charge density by the ions was then taken as the difference in charge density between that of a suspension

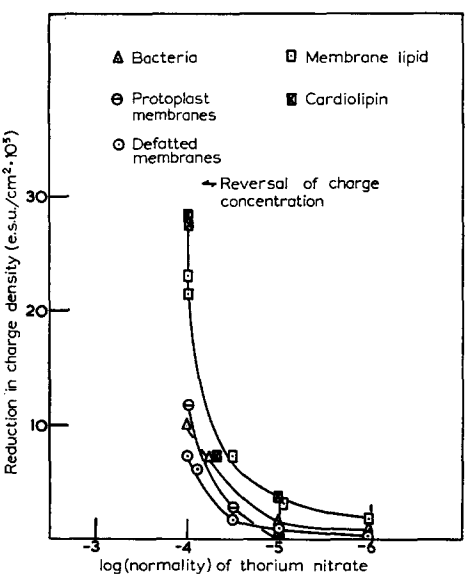

Fig. 2. Reduction in charge density of suspensions of cardiolipin and bacterial derivatives by thorium nitrate.

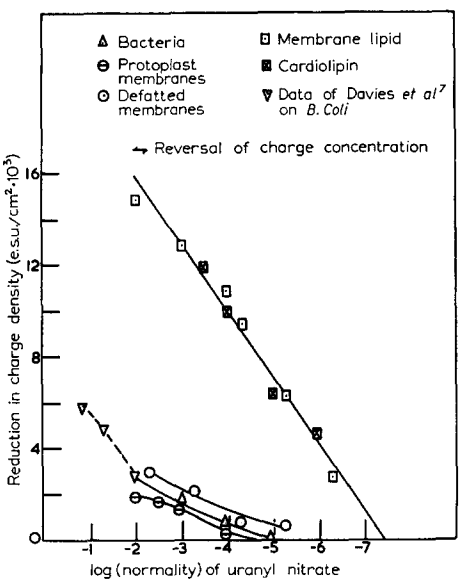

Fig. 3. Reduction in charge density of suspensions of cardiolipin and bacterial derivatives by uranyl nitrate.

containing either uranyl nitrate or thorium nitrate at a given concentration and that of a control suspension under identical conditions except for the absence of the cation under consideration. The reductions in charge density of the various bacterial derivatives and cardiolipin are plotted against log (normality) of thorium nitrate and uranyl nitrate respectively in Figs. 2 and 3, together with some comparable data of DAVIES, HAYDON AND RIDEAL[7] on *Bacillus coli* and uranyl ions. The curves show that similar reductions of surface charge density are obtained with bacteria, protoplast membranes and defatted protoplast membranes. With the membrane lipid and cardiolipin however, the effects are greater, especially those with uranyl ions. The reversal of charge concentrations of both ions are similar for each of the five types of suspension examined and these too are indicated in Figs. 2 and 3.

In choosing, for purposes of comparison of the systems, any given value of the

reduction in charge density, the relative concentration of the ions can vary with the value of charge density reduction selected. This is particularly so with thorium nitrate. For reasons discussed later, it is preferable to use reductions in surface charge density which are small. Further, in order to allow for valency effects and to compare concentrations at equal numbers of ions adsorbed, the comparisons have been made at reductions in charge density which are proportional to the valency of the ion under consideration. A charge density reduction of $1 \cdot 10^3$ e.s.u./cm^2 was chosen for uranyl ions and a reduction of $2 \cdot 10^3$ e.s.u./cm^2 for thorium ions. The concentration of ions producing these reductions in surface charge density is defined as the "isosteric ion adsorption concentration".

The isosteric ion adsorption concentrations are given in Table II. These demonstrate that uranyl ions are extremely potent in producing small reductions in charge density of purely phosphate type colloids, such as cardiolipin and in the protoplast membrane lipid. The difference with thorium ions is not nearly so great, although it too has lower values for the phosphate type. By contrast, the use of reversal of charge concentrations on the present examples fails to differentiate between them.

TABLE II

ISOSTERIC ION ADSORPTION CONCENTRATIONS OF ELECTROLYTES ON CELL FRACTIONS

System	*Isoteric ion adsorption concentration (N)*	
	Th^{4+}	UO_2^{2+}
Bacteria	$1.7 \cdot 10^{-5}$	$2.8 \cdot 10^{-4}$
Protoplast membranes	$2.2 \cdot 10^{-5}$	$6.3 \cdot 10^{-4}$
Defatted membranes	$4.0 \cdot 10^{-5}$	$1.0 \cdot 10^{-4}$
Membrane lipid	$2.2 \cdot 10^{-6}$	$8.3 \cdot 10^{-8}$
Cardiolipin	$2.2 \cdot 10^{-6}$	$8.3 \cdot 10^{-8}$

DISCUSSION

The results presented enable a number of conclusions to be reached concerning the membrane architecture of *M. lysodeikticus* and the ionogenic groups present in its surface.

The pK values derived from the pH–mobility relationships of the protoplast membranes before and after lipid extraction agree closely with those quoted for carboxyl and amine groups in a number of amino acids[5]. Alanine, glutamic acid, glycine and lysine are known constituents of the cell walls of *M. lysodeikticus*[6]. These and several other amino acids have been shown[3] to be present also in the non-lipid fraction of the protoplast membrane. Carboxyl groups with similar pK values may originate also in polysaccharide components[7]. Such components have also been demonstrated in *M. lysodeikticus* in the bacterial cell walls[6] and the defatted protoplast membranes[3]. Precise comparisons with known compounds are not possible because it is not known how the molecular components are linked in the macrostructure of the cell derivatives. However, it appears likely that the electrophoretic behaviour of these biological fractions is controlled by the ionization of carboxyl and amino groups in the surface. Although the membrane lipid has a lower pK than cardiolipin, the acidity of the phosphate group is known to vary with the degree of

esterification. The membrane lipid has been shown[3] to contain at least three fractions, the major component of which reacts as a phosphatidic acid. Thus the character of the mobility–pH curve of the membrane lipid is attributed to the presence of phosphate groups. The permanent effects of extremes of pH on the cell membranes of *M. lysodeikticus*[8] appear to occur at a deeper level not contributing to the electrokinetic properties of bacteria or protoplast membranes, since these, held at either pH 2.3 or 10.0, resumed the usual mobility of controls on being returned to neutrality. Similar results have been reported with *Aerobacter aerogenes*[9].

In previous work to elucidate the nature of unknown charge groups by the use of ionic reversal of charge spectra[1], the phosphate colloid models were all phosphatides *e.g.*, lecithin, containing basic groups. For these the reversal of charge concentrations of both uranyl and thorium ions are low (*ca.* $10^{-4}N$). For carboxyl colloid models, however, the reversal of charge concentration of uranyl ions is much higher (approx. 10^{-1} to $10^{-2}N$). The cardiolipin investigated here had a reversal of charge concentration of $10^{-4}N$ for thorium ions and 10^{-2} N for uranyl ions, values previously thought to be more characteristic of carboxyl or possibly sulphate type colloids, whereas cardiolipin is a complex phosphatidic acid without basic groups[10]. The use of reversal of charge concentrations of ions as a criterion of charge group type appears unreliable for three reasons. (a) the coexistence of other types of charge groups can result in abnormal sensitivity to some ions, (b) a colloid with a surface of high charge density will have a higher reversal of charge concentration for a particular ion than the same type of surface with a lower charge density, (c) steric effects are likely to be of importance, particularly in the last stages of the neutralization of charge. The use of isosteric ion adsorption concentrations as defined earlier, utilizing small equal reductions in charge density as a basis for comparison, avoids these difficulties and is much more suitable as a basis for comparison within the systems used here. However, it could not be claimed at this stage that it constitutes a generally valid test for charge group type.

It is usually considered that the plasma membrane of cells consists of a more or less continuous bimolecular layer of oriented lipid molecules on which are adsorbed layers of protein[11]. The evidence for this is almost entirely indirect. The present results provide some experimental support for the general picture as it applies to the bacterial protoplast membrane.

The electrophoretic properties of protoplast membranes are very similar to those of the defatted membranes. This is evident from the pH-mobility curves and the effects of uranyl and thorium ions summarized in Table II. Such minor differences as do exist probably arise from very slight contamination of the defatted membranes with traces of lipid. On the other hand, these properties are entirely dissimilar from those of the membrane lipid. Hence there is strong evidence that the surface of the protoplast membrane does not contain lipid but is similar in nature to the protein–carbohydrate fraction of the membrane. The similar electrophoretic behaviour of protoplasts and protoplast membranes enables these conclusions to be transposed directly in terms of the structure of protoplasts. This study of the electrophoretic properties of the bacterial derivatives thus indicates that the outer layer of the *M. lysodeikticus* protoplast membrane is composed of protein, possibly together with polysaccharide, and that the lipid component must occupy an internal position deeper within the membrane.

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REFERENCES

1 H. G. BUNGENBERG DE JONG, in *Colloid Science*, Editor KRUYT, Elsevier, Amsterdam, Vol. II, chapter 9, 1949.
2 C. R. BRINTON AND M. A. LAUFFER, *Electrophoresis*, Editor A. P. BIER, New York 1959, p. 427.
3 A. R. GILBY, A. V. FEW AND K. MCQUILLEN, *Biochim. Biophys. Acta*, 29 (1958) 21.
4 A. D. BANGHAM, R. FLEMANS, D. H. HEARD AND G. V. F. SEAMAN, *Nature*, 182 (1958) 642.
5 E. J. COHN AND J. T. EDSALL, *Proteins, amino acids and peptides*, REINHOLD, New York, 1943, chapter 20.
6 M. R. J. SALTON, *Biochim. Biophys. Acta*, 10 (1953) 512.
7 J. T. DAVIES, D. A. HAYDON AND E. K. RIDEAL, *Proc. Roy. Soc., (London) B*, 145 (1956) 375.
8 A. V. FEW, M. J. FRASER AND A. R. GILBY, *Biochim. Biophys. Acta*, 24 (1956) 306.
9 J. H. B. LOWICK AND A. M. JAMES, *Biochem. J.*, 65 (1957) 431.
10 M. C. PANGBORN, *J. Biol. Chem.*, 168 (1947) 351.
11 J. F. DANIELLI, *Cytology and Cell Physiology*, Editor BOURNE, O.U.P. Oxford 1951 p. 150.

Biochim. Biophys. Acta, 38 (1960) 130–136

ÉTUDE DU "RÉTROCONTROLE" DE SYNTHÈSES D'ENZYMES PAR DES ACIDES AMINÉS AU COURS DE LA CROISSANCE DE *PROTEUS MORGANII**

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SUMMARY

Feedback control on synthesis of enzymes by amino acids during growth of P. morganii

The kinetics of growth of *Proteus morganii* shows evidence for a feedback control on the synthesis of enzymes by cysteine and methionine.

These repressions are expressed by lags in the growth and by "diauxies" and "triauxies".

The pool of repressors interferes with the expression of the repression.

INTRODUCTION

Lors de son étude sur la croissance bactérienne, MONOD[3] a montré que lorsqu'on fait croître des bactéries en présence de deux sucres dont l'un (a) est métabolisé par

* Une partie de ce travail a fait l'objet de notes préliminaires[1,2].